PROJECT AUTHORIZATION NO. HWY-2004-09

under

MASTER AGREEMENT FOR RESEARCH AND TRAINING SERVICES BETWEEN THE NORTH CAROLINA DEPARTMENT OF TRANSPORTATION AND NORTH CAROLINA STATE UNIVERSITY ON BEHALF OF THE INSTITUTE FOR TRANSPORTATION RESEARCH AND EDUCATION (Contract No. 98-1783)

Project Title: An Evaluation of Hemolymph Extraction as a Non-Lethal Sampling Method for Genetic Identification of Freshwater Mussel Species in Southern North Carolina

Formal Statement of Work: See attached proposal from UNC - Wilmington

Period of Performance: July 1, 2003 – June 30, 2005

Budget Authorization: $41,348 (FY'03-04)
$35,708 (FY'04-05)
$77,056 (TOTAL)

Property to be Furnished by the Department: None

Key Personnel: Dr. Ami E. Wilbur and Dr. Michael A. McCartney, UNC-Wilmington

Project Monitor: Mr. Mustansir Kadibhai, P.E.

Additional Terms and Conditions: Research Project Guidelines as posted on ITRE's website at *http://itre.ncsu.edu/research/ongoingguidelines.htm.*

IN WITNESS WHEREOF, the parties hereto have executed this Project Authorization as of

_____, 2003.

NORTH CAROLINA STATE UNIVERSITY NORTH CAROLINA DEPARTMENT OF TRANSPORTATION

BY:_____ BY:_____
Principal Investigators

BY:_____
N. C. State University

BY:_____
Director of ITRE

FY 2004 NCDOT Research Proposal

Amount Requested: $67,056 (total for two years)

Project Title: An Evaluation of Hemolymph Extraction as a Non-Lethal Sampling Method for Genetic Identification of Freshwater Mussel Species in Southeastern North Carolina

Purpose of Proposal: Develop non-lethal sampling techniques for genetic analysis of mussels

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Submitted: 25 April 2003

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STATEMENT OF WORK

A. Introduction

North America boasts the highest diversity of freshwater mussel species anywhere. Almost three-quarters of these species, however, are at risk of extinction (Stein and Chipley 1996), and like many freshwater organisms, are particularly threatened by construction of roads, bridges, and residential and commercial structures. Of the 47 freshwater mussel species reported from North Carolina waters, >30 are considered to endangered, or are species of special concern to state and federal agencies (NC Mussel Atlas: http://www.ncwildlife.org/pg07_WildlifeSpeciesCon/pg7b1a.htm; Legrand et al. 2001). State and federal regulators, therefore, are increasingly asking agencies like the NC Department of Transportation to document whether a threatened or endangered freshwater mussel exists at a proposed construction site. And since the presence of an endangered species can halt or greatly increase the cost of a construction project, methods for identifying and distinguishing freshwater mussel species at field sites are therefore in great need.

B. Background

Biologists have generally relied upon physical characteristics to delineate species of freshwater mussels, but this approach is notoriously difficult to implement and likely unreliable for distinguishing taxa. Even distantly related species can show very similar external characteristics, and shell traits are known vary in response to environmental conditions (Mulvey and Kandl 1998). Molecular genetic methods of identification provide a powerful alternative. Sequences of both mitochondrial and nuclear DNA regions have been used very effectively to distinguish species and evaluate the genetic relationships among disjunct populations of freshwater mussels (*e.g.* King et al. 1999; Mulvey et al. 1997; Roe et al. 2001). There are logistical problems associated with genetic methods, however. First, mortality from tissue sampling by biopsy or by collecting whole animals prevents biologists from sampling populations at risk and hinders development of methods for distinguishing endangered from non-endangered populations. Secondly, methods that rely upon DNA sequencing require expensive equipment and materials and trained personnel that limits their applicability by NCDOT and other agencies. This proposal is aimed at overcoming these logistical limitations on the implementation of genetic methods for identifying freshwater mussel species in North Carolina waters.

Recent work with captive freshwater mussel populations (Gustafson et al. 2001) showed that removal of 0.5cc of hemolymph from the adductor mussel of *Elliptio complanata* did not result in high levels of mortality; survival was 90% after 13 weeks. Similar work with marine mussels showed that mortality of animals from which hemolymph was obtained was not significantly different from controls up to a year after sampling. The latter studies, moreover, reported that the extracted hemolymph performed similarly to solid tissue samples with respect to yielding DNA suitable for genetic analysis (Yanick and Heath 2000). This research indicates that drawing hemolymph from mussels produces little or no mortality, and is therefore a viable

method for non-lethal sampling that deserves evaluation in natural populations of endangered or threatened species. Hemolymph also appears to be a good candidate source of PCR-amplifiable DNA in marine bivalves, but this requires confirmation for use with freshwater mussels. Initial work by NCDOT funded researchers at NCSU (described by T. Savidge in NCDOT's FY 2004 Research Idea EN-05) shows that hemolymph extraction from freshwater mussels is a viable non-lethal sampling technique. Survival rates after hemolymph extraction have so far proven to be excellent, and "…preliminary studies…indicate that the cells in the sample can potentially be used to conduct genetic analysis."

Our own preliminary studies, performed in collaboration with J. Johnson and J. Alderman of North Carolina Wildlife Resources Commission (NCWRC), demonstrates the great potential that DNA sequence analysis holds to resolve taxonomic problems and to provide a method for genetic identification of freshwater mussels from North Carolina waters. With the assistance of Johnson and Alderman and their expert identifications of species based on shell characters, we assembled a collection of 4 putative species of *Lampsilis* and 1 species of *Elliptio* from the Neuse, Tar, and Pee-Dee drainages in North Carolina, the latter including Lake Waccamaw and the upper Waccamaw River. Our work has so far focused on Lake Waccamaw due to our interest in its endemic fauna. In particular we have focused on the genus *Lampsilis*, which contains several threatened species widespread in North Carolina, as well as several geographic populations of uncertain taxonomic status and of concern to conservation biologists.

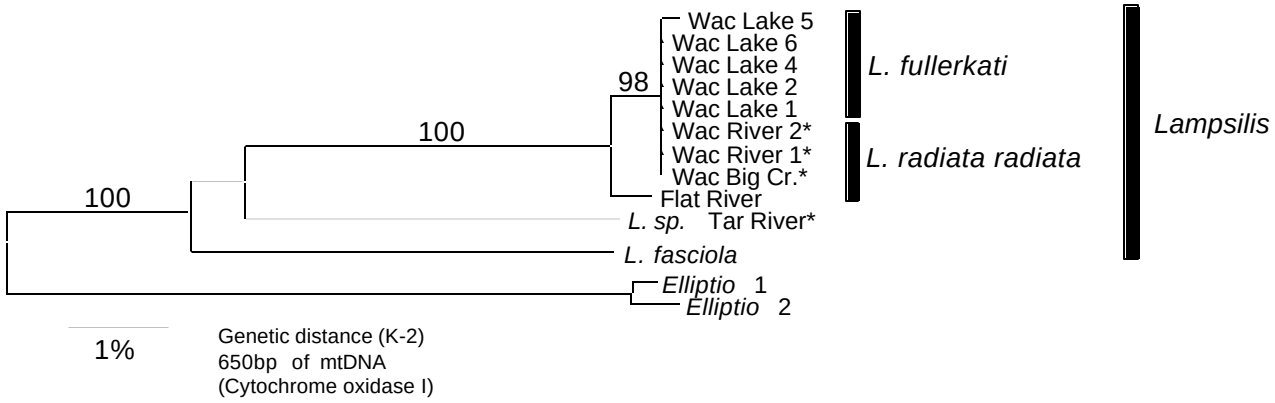

Fig. 1. Preliminary phylogenetic analysis of mtDNA sequences: *Elliptio* 1 and 2 were collected by M. McCartney from the Black River (Cape Fear drainage); *L. sp.* is a *Lampsilis,* species uncertain, collected from the Tar River by J. Johnson and J. Alderman (JJ & JA) of NCWRC. The remaining *Lampsilis* include a confirmed *L. radiata radiata* from the Flat River (Neuse drainage), a suspected *L. radiatia radiata* from Lake Waccamaw/Big Creek, two suspected *L. radiata radiata* from the upper Waccamaw River, and five *L. fullerkati* from Lake Waccamaw. Waccamaw Lake and River samples were collected by the authors and by JJ & JA. The *Lampsilis fasciola* sequence is from GenBank (Accession # AF156520). The tree was constructed by neighbor-joining (Saitou and Nei 1987) using distances corrected using Kimura's (1980) 2-parameter model. Numbers above branches are bootstrap support values = 70 (1000 replications). *Asterisks denote uncertain species designation.

Conclusions that can be reached from our preliminary phylogenetic analysis (Fig. 1) include: (1) Waccamaw River animals identified as putative *L. radiata radiata*, based on morphological features, are a distinct species or subspecies, (2) they are not distinguishable genetically from the morphologically distinct endemic morphotype *L. fullerkati*, suggesting that Waccamaw River and Lake forms may be synonymous (3) the Waccamaw animals we sampled are not *L. radiata radiata*, but are close relatives, and (4) the undescribed specimen from the Tar River is clearly a *Lampsilis*, but its species identification awaits further analysis.

Lake Waccamaw holds 11 freshwater mussel species (Stansbery and Clench, 1978; Porter and Horn, 1983), and the sampling design detailed in this proposal will allow us to obtain DNA sequences from several individuals of several of these species. This will allow us to determine the taxonomic status of the putative endemics in the genera *Lampsilis* and *Elliptio,* and more generally, to characterize the species diversity of the lake. The large population densities of mussels in the lake provide an unparalleled opportunity for us to refine non-lethal methods of hemolymph sampling with minimal risk, and the methods so developed will allow us to sample the rarer species in the lake and elsewhere. The diversity of taxa found in Lake Waccamaw include relatively common species of the genera *Lampsilis, Elliptio*, and *Leptodea* as well as one federally endangered, one state endangered, and three state threatened species found elsewhere in the state. Hence the methods we develop will be broadly applicable, resulting in the development of genus- and species-specific markers for genetic identification of several mussel species that may be impacted by proposed NCDOT projects. Further sampling outside Lake Waccamaw, as outlined in the second year will expand our genetic database for species found in North Carolina waters and increasing our ability to assesses the distinctiveness of mussels sampled elsewhere in the state.

C. Definition of Need

ITRE's call for preproposals for FY 2004 (**EN-05: "Development of Field and Laboratory Standard Operating Procedures for Genetic Identification of Freshwater Mussel Species"**) clearly outlines the need for our project. State and federal regulatory agencies will increasingly call upon DOT biologists to determine whether the site for a proposed construction project is home to an endangered or threatened species of freshwater mussel. Biologists conducting surveys currently depend on species identification based primarily on examination of diagnostic shell characteristics, which are difficult to discern and may be unreliable, even in the hands of experts. Genetic identification offers a powerful alternative, but creates new challenges that this proposal aims to help solve. First, we will overcome the need to sacrifice animals or to obtain tissue biopsies, either of which is clearly not a viable approach to sampling a threatened population. The methods we develop for obtaining, storing, and processing hemolymph for DNA extraction will allow even the rarest species to be sampled with little or no risk of mortality. Secondly, genetic methods based on DNA sequence analysis require equipment and expertise not generally available to NCDOT and other state agencies. However, the end result of our work will be standard

operating procedures that will involve DNA extraction, PCR, restriction digestion, and agarose gel electrophoresis that will greatly simplify genetic analysis as well as establish a standard set of procedures that will ensure consistency across the state. Standardization of identification procedures will streamline the process, and each additional analysis, if added to a common database, will further our ability to quickly evaluate the genetic distinctiveness of mussel populations. Such methodologies could substantially facilitate the evaluation process of potential impacts from proposed NCDOT construction projects.

D. Research Objectives

(1) To obtain hemolymph samples from several freshwater mussel individuals from several species collected at multiple sites in Lake Waccamaw.

(2) To monitor the survivorship of sampled individuals for several weeks to evaluate the effects of hemolymph withdrawal on survivorship under field conditions.

(3) To develop methods for hemolymph transport, storage and processing to allow extraction of PCR-amplifiable DNA from this tissue.

(4) To PCR amplify and sequence the DNA of 2 mtDNA regions and 1 nuclear DNA region from a subset of the individuals sampled.

(5) To analyze the DNA sequences phylogenetically to define monophyletic groups (i.e. species and subspecies), and to use these analyses to design genus- and species-specific restriction endonuclease digestion assays.

(6) To apply methods developed in (5) to new locations, we will sample and sequence the DNA, then perform diagnostic restriction digests on selected animals from adjacent and more distant collection sites in North Carolina.

E. Research Methodology and Itemized Tasks

Year 1

<u>Objectives 1 and 2: Collection of animals from Lake Waccamaw and field evaluation of non-lethal sampling for genetic analysis.</u>

We propose to provide a test case of hemolymph sampling for genetic analysis, using specimens collected in Lake Waccamaw in Columbus County, North Carolina. The lake is an excellent site for the proposed study, because it contains relatively dense assemblages of a diversity of mussel species from genera that are broadly distributed in NC, as well as some endemic populations of uncertain taxonomic status that are currently under consideration for protection (see North Carolina Mussel Atlas). Hence the genetic methods of identification we develop can be tested on the lake's full taxonomic range, from geographic populations to putative endemic species to well-differentiated species and related genera.

We propose to use hemolymph sampling to non-lethally sample mussel populations in Lake Waccamaw and monitor short-term survival of sampled individuals. Specifically, we will sample 3 sites in Lake Waccamaw (State Park, northeast shore, and near dam), and collect along 3 transects laid at each of these sites. Short-term monitoring of survivorship from hemolymph extraction will be carried out at 3 transects

laid at the State Park site only. We have chosen this site for logistical reasons—it is easiest to access and has the lowest potential for problems due to tampering because of the proximity of Park personnel. Because the mortality associated with field sampling of hemolymph is yet unknown, we propose to complete the transects and monitoring at the State Park site first, and then execute sampling at the other sites. We anticipate minimal mortality, based on the PI's experience with marine bivalves, and consequently have scheduled the sampling at the other two sites for late September (see time line).

Transects will be run perpendicular to the shoreline, and 30 meters in length, at a nearshore (= 0.5 m depth), mid (0.5 - 1m), and deep (1 - 2m) location at each site. We will collect along an ~ 1m swath along the transect all mussels encountered, and collect hemolymph from a quasi-random subset of individuals. All mussels will be measured, a preliminary species identification made, and all mussels will be digitally photographed to allow reconciliation of genetic patterns with gross morphology.

Fifteen individuals of each of the most abundant species (*Elliptio waccamawensis, Leptodea ochracea,* and *Lampsilis fullerkati*: Porter and Horn, 1983) will be randomly selected with regard to body size for hemolymph extraction; and one-half of the total number of representatives of other species will also be sampled. Hemolymph will be drawn using a fine-gauge syringe from adductor muscle tissue, then placed into sealed tubes on ice (see below). Hemolymph-sampled mussels, and an equivalent number of those of each species not chosen for hemolymph extraction, will then be marked and returned to 30 m^2 mesh enclosures. We will place each enclosure 10 cm into the substrate and extending 0.25 m above the substrate, and each located at the site of sampling to facilitate the location of sampled individuals over time. Animals in enclosures will be checked after 1 week, 4 weeks and 8 weeks to quantify mortality of both hemolymph-sampled and control individuals. Nearshore and mid-depth transects can be checked by wading along the transect; for deep enclosures we will use SCUBA. Our procedures will result in enclosures being located at the same site of encounter of the animals, and specimens will be replaced at their original density. Enclosure size will be modified from the proposed 30 m^2, if necessary, to obtain the desired sample sizes, but we will not alter the density of animals in the enclosures from those encountered during the collection.

The sampling scheme outlined above will provide hemolymph samples for genetic analysis. We do not intend to target particular species (there are reportedly ~11 species of mussels in the Lake. We will sample quasi-randomly to provide a field test that is not species specific, and yet one that maximizes our encounter probability of rarer species, while at the same time providing a representative sample of the range of body sizes and ages of more abundant species. While different species may prove to have distinct tolerances to the removal of hemolymph, it is our desire to develop methodologies that do not require species identification prior to sampling. Further, the random sampling will provide us with a relatively unbiased sampling of the genetic resources in the Lake.

The tag number and general condition of each animal will be recorded on each sample date. The rate of failure to recapture marked animals (*e.g.* to predation or escape) will be calculated and compared across species and transects by non-parametric tests. Mortality incidence for each species will be tallied and mortality for

controls will be compared to mortality of hemolymph-sampled animals by G-tests (Sokal and Rohlf, 1981).

Objectives 3, 4 and 5: Genetic Identification of Lake Waccamaw mussels

We propose to amplify by PCR selected regions from both the nuclear DNA and mtDNA. DNA sequences of these regions will be obtained from all mussel individuals. DNA sequence data will next be subjected to phylogenetic analysis to determine phylogenetic status of putative species, and to aid in design of identification assays. Sequence alignments and phylogenetic analyses will be used to develop diagnostic markers for species identification using restriction endonuclease digestion of PCR products. We will develop identification assays based on restriction fragment length polymorphisms that are capable of providing genus and species-level identification, as well as identification of any other Evolutionarily Significant Units (ESU's: Moritz, 1995) that are indicated by the data. Specifically, we will explore the possibility of using suites of enzymes to identify distinct population segments of the more abundant or widely occurring species. This will allow us to design rapid and economical methods for species identification that rely on (1) preparation of DNA templates from hemolymph, (2) PCR amplification, followed by (3) restriction enzyme digestion, and (4) analysis using agarose gel electrophoresis. Application of the methods we develop will greatly reduce the cost of analysis and make it more readily available to state laboratories.

Throughout our study, we will evaluate methods for transport and storage of hemolymph, by using samples from the most abundant species, by comparing DNA recovery and quality from undiluted hemolymph to that obtained from hemolymph diluted in isotonic phosphate-buffered saline (PBS) at the time of collection. Hemolymph DNA will be extracted using standard methods in the Wilbur and McCartney laboratories and quantified spectrophotometrically and run on agarose gels for qualitative assessment. All DNA extracts and hemolymph samples will be stored at -80 °C at UNCW.

We will PCR-amplify DNA from two mtDNA regions (COI and the 16s ribosomal DNA) and one nuclear region (the internal trascribed spacer (ITS) regions of nuclear ribosomal RNAs) in order to evaluate the ability of the various marker regions to discriminate among the various species. Primers designed for use with unionids are published (Mulvey et al. 1998) and we have preliminary data indicating the successful use of one of these regions (CO I: Fig. 1) with mussels in the genus *Lampsilis* (Wilbur and McCartney unpublished). Amplicons will be sequenced using Applied Biosystems (ABI) kits and analyzed on our ABI 3100 automated genetic analyzer at the Center for Marine Science at UNCW. Sequences will be edited and checked for sequencing and reading frame errors, and aligned using ClustalW (Thompson et al. 1994). Best-fitting models of molecular evolution will be selected using hierarchical likelihood ratio tests available in *ModelTest 3.06* (Posada and Crandall 1998), and phylogenetic analysis will be performed using *PAUP* 4b10* (Swofford 2002). Monophyletic groups will be inspected to determine the morphological species status and geographical location of any ESU's so identified. Fixed nucleotide sites that define these groups will be inspected to select inexpensive restriction endonucleases that can be used to distinguish species without the need for DNA sequencing.

These data and analyses will be used to develop efficient and economical assays that reveal restriction fragment length polymorphisms (RFLPs) of PCR products that can rapidly differentiate among taxonomic groups. We have accomplished similar types of marker development for the identification of marine species from a wide range of taxa (eg. fish, larval crabs and bivalves).

Year 2

Objective 6: Application of non-lethal identification assays

Using the assays developed in Year 1 (and assuming we will observe minimal mortality associated with hemolymph extraction), we will extend our sampling to provide a more comprehensive characterization of the Pee Dee drainage with emphasis on the Waccamaw River. Specifically, we would like to focus on two genera of mussels found in the Waccamaw system (*Lampsilis* and *Elliptio*). We propose to sample a maximum of 15 mussels per genus per site at two sites in the Waccamaw River, one site in Big Creek (a major stream feeding into the SE shore of Lake Waccamaw) and one site in the Lumber River system (Drowning Creek, near the Hoke County/Scotland County line). This survey will provide valuable information regarding the occurrence of two putative endemic Lake species (*Lampsilis fullerkati* and *Elliptio waccamawensis*) as well as their congenerics (*L. cariosa, L. radiata* and *E. folliculata*) and will produce base line genetic data on the degree of differentiation among drainages for these taxa.

A modest number of additional samples from other drainages will be incorporated if funds and samples are available. We have worked previously with Judy Johnson and John Alderman (NCWRC, Nongame & Endangered Wildlife Program) and continued to rely on their expertise to guide our sampling of critical populations of mussels. It is important to note that, as new putative species are added to our analysis, our ability to design PCR/RFLP assays that will be broadly applicable in North Carolina will continue to improve. Each new added species, however, requires the generation of multiple PCR products, DNA sequences and considerable time investment. Nevertheless, the end product of future work and continued funding would be a comprehensive set of rapid and inexpensive assays that could be employed at virtually any site in North Carolina at which a construction project is proposed.

Our sequence data will be deposited in GenBank for public access in a timely manner along with manuscript and report preparation. However, we also plan to initiate collaboration with biologists at NCDOT and NCWRC for the purposes of developing a public-access online genetic database for management and monitoring of freshwater mussels. Each of our samples will be stored at UNCW, and each will be cross-referenced to a digital photograph, GPS coordinates, and for a subset of these, multiple DNA sequences will also be archived. As stated above, as genetic data on North Carolina mussels continues to be accumulated, improvements in taxonomy and the definition of ESU's will accrue. In addition, design of multiple-enzyme assays for rapid identification will become more challenging, but more comprehensive in scope for the state. With continued funding, we would advise and help facilitate the construction of a shared depository of genetic information on freshwater mussels. An excellent place to start would be the outstanding online Freshwater Mussel Atlas prepared by J Alderman and others at NCWRC and other agencies (http://www.ncwildlife.org/pg07_WildlifeSpeciesCon/pg7b1a.htm).

F. Significance of Proposed Work

Based on initial findings at NCSU and published reports, we expect little mortality from hemolymph extraction, but now we will be able to establish this with carefully controlled experiments under field conditions at a location that is perfectly suited to this study. We expect DNA extractions from hemolymph to produce reliable PCR amplifications, and one of us (A. Wilbur) has recently being using hemolymph very successfully as source of DNA for PCR amplification and sequencing with scallops, a notoriously difficult animal from which to obtain amplifiable DNA from other tissues. We are confident that methodologically, accomplishing the molecular aspects of the proposed study will be straightforward, up to the point of design of the RFLP assays. We expect that the sampling and molecular methodologies that we develop will be useful to NCDOT biologists and others in the state of North Carolina and elsewhere.

Forecasting the results of our phylogenetic analysis, and therefore the design of the RFLP analyses, however, is less straightforward. We fully expect to encounter many unexpected results, given that unionid systematics and morphological taxonomy is fraught with difficulties. Based on our initial analysis of *Lampsilis fullerkati*, on initial work with endemic fishes (McCartney unpublished), and on considerations of the extremely young age of the lake, we expect important but challenging outcomes with respect to Lake Waccamaw endemic fauna. For example, we may continue to find that animals from the upper Waccamaw River and Lake Waccamaw together constitute a distinct clade within a genus, without animals from the Lake itself being reciprocally monophyletic. This may suggest subspecies or other status for the headwaters of the Waccamaw (including the lake) but might question the status of the lake forms (some of which, like *E. waccamawensis,* are *very* distinct morphologically) as separate endemic species. We expect several other similar issues as the study would expand geographically.

There are practical implications of this for our recommended protocols for performing mussel identifications based solely on molecular markers. We can only design "diagnostic" RFLP's to recognize fixed nucleotide substitutions; *i.e.* we can only recognize and distinguish phylogenetic species. Waccamaw endemics, for instance, that are too young to have achieved monophyly but still are morphologically (and perhaps reproductively) distinct will not be recognized as distinct using our assays. Nevertheless, the approach we recommend is in keeping with current conservation philosophies that place a great deal of emphasis on evolutionary and phylogenetic distinctiveness (see Moritz 1995 and references cited therein). Since morphology of freshwater mussels is poorly correlated with genetic relationship and since it is often very plastic, we believe our approach to be a promising alternative for estimating the distinctiveness of a population of animals and its prospects for long-term conservation. Our approach also offers a way to develop markers for identification that can be objectively established, regulated, and carried out by state and federal agencies.

G. Recommendations for Implementation and Technology Transfer

Our work will result in a recommendation for standard procedures to be used in genetic identification of freshwater mussel species in North Carolina. Methods previously implemented for non-lethal sampling of hemolymph under laboratory conditions will be refined to allow their use in the field. The primary products will be protocols for preservation and storage of hemolymph, for DNA extraction or other preparation of hemolymph for genetic analysis, and protocols for analyzing species-diagnostic molecular genetic markers. These methods will be rapid and cheap enough to be utilized by contract laboratories. Our secondary product will be a DNA sequence database that can, in the future, form the core of a genetic inventory of freshwater mussel biodiversity in North Carolina and across the southeastern US. Free public access to this database through the web could be the product of future work.

Use of products by NCDOT

We envision that NCDOT can use the protocols here developed, in consultation with a contract lab, to obtain survey information. The customers using the products will be NCDOT biologists charged with survey duties. Field biological technicians could conduct these surveys, and take the hemolymph samples and photographs much as we propose, then send the hemolymph (preserved and transported using the protocols we develop) to contract labs. Field technicians would not need to be experts in freshwater mussel taxonomy based on external physical characters (which can be unreliable in any case). The information gained from the surveys would allow NCDOT to determine whether endangered mussel species are present at the site of a proposed construction project, and to do this without inflicting harm on the animals. Our RFLP assays would, moreover, lower the cost of analysis by the contract labs, and allow the work to be done in many more laboratories than can currently purchase and support a sequencing facility. Again, the scenario we envision will not take place without systematic design of molecular procedures, designed specifically for the species present in North Carolina, such as we propose to develop.

H. Proposed Work Schedule

We provide a schedule for research tasks below. General methods for each task are described in Section E.

Date	Tasks
July - September 03	Sample and monitor survivorship of hemolymph-sampled animals along State Park Transects
October 03	Sample northeast shore and near shore transects
November-May 04	DNA extraction and PCR method development begins Sequence DNA's, sequence analysis and design of RFLP assays
June 04 — August 04	Collection from adjacent and other drainages, DNA extraction, PCR Sequence DNA's,
October 04— April 05	Sequence analysis, refinement of RFLP assays for genetic identification of all taxa encountered
May — June 05	Finish analyses; report and manuscript preparation

I. References

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Education

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Professional Experience

8/99-present	Assistant Professor, Department of Biological Sciences, UNCW
10/97-7/99	Postdoctoral Research Associate, Florida Marine Research Institute
01/96-6/97	Postdoctoral Research Associate, University of Delaware
07/94-12-95	Research Assistant, University of Delaware
09/93-6/94	Assistant Professor, Department of Biology, Salisbury State University

Recent Publications

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7. Wilbur, A.E. and P.M. Gaffney. 1997. A genetic basis for geographic variation in shell morphology in the bay scallop, *Argopecten irradians*. Marine Biology 128:97-105

Current Projects

1. 03/04 ODRP-SeaGrant $410,570 “Development of Single Nucleotide Polymorphism (SNP) Markers in the Eastern oyster for Genetic Improvement and Stock Enhancement” (co-PI)
2. 3/03-2/04 $19,850 CICEET “Biological treatment of effluent from an intensive marine finfish recirculating aquaculture facility by cultivation of marine microalgae and bivalves” (co-PI)
3. 7/03-7/05 $67056 NCDOT “An evaluation of Hemolymph Extraction as a non-lethal Sampling method for Genetic Indentification of Freshwater Mussel Species in Southeastern North Carolina (co-PI)
4. 4/01-6/03 $40,198 Fishery Resources Grant, NC Sea Grant “Evaluating the reproductive output of oyster aquaculture: do commercial grow out operations contribute to local recruitment” (PI)
5. 7/01-6/03 $31,989 North Carolina Sea Grant “A new method for the evaluation of spatial and temporal dispersal patterns of blue crab (*Callinectes* spp.) larvae in the Cape Fear River Plume” (PI)
6. 05/02-04/03 $31,538 National Fish and Wildlife Foundation “ Taxonomic status of the potentially endangered Key silverside (*Menidia conchorum*) in southern Florida, with recommendations for conservation” (co-PI)
7. 4/02-4/03 $26,048 NC Fisheries Resource Grant “Evaluation of spatfall in the Cape Fear estuary” (co-PI)
8. 07/02-02/04 $206,753 2001 Saltonstall-Kennedy Program “Bay scallop (*Argopecten irradians*) population restoration on the west coast of Florida” (co-PI)

Collaborators (1999-2002)

1. **Collaborators**: Co-authors/co-PI’s (listed alphabetically) TD Alphin (UNCW), WS Arnold (Florida Marine Research Institute), JC Bailey (UNCW), TM Bert (FMRI), N Blake (University of South Florida), PAX Bologna (Fairleigh Dickinson University), R Carnegie (VIMS), SC Cary (University of Delaware), DO Conover (State University of New York, Stony Brook), DW Freshwater (UNCW), TE Lankford, Jr (UNCW), K Leber (Mote Marine Laboratory), W. Loftus (FIU), B. Lockwood (FWS), MA McCartney (UNCW), MH Posey (UNCW), K Reece (VIMS), S.Seyoum (FMRI), W Watanabe (UNCW)

2. **Graduate Advisors**: PM Gaffney (University of Delaware), TJ Hilbish (University of South Carolina)

3. **Post Doctoral Advisors**: PM Gaffney (University of Delaware), TM Bert (Florida Marine Research Institute)

4. **Graduate Advisees**: Rachel Cox Sackett, Amanda Myers, Steven Truesdale, Russ Peterson

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A. Professional Preparation

Ph.D State University of New York at Stony Brook, 1994 (Ecology and Evolution)
M.S. Case Western Reserve University, 1988 (Environmental Sciences)
B.S. Florida State University, 1981 (Biological Science)

B. Appointments

01/00-	Assistant Professor, Department of Biological Sciences, UNCW
01/99-12/99	Postdoctoral Research Associate, Department of Biological Science, Florida State University
09/95-12/98	NSF/Sloan Molecular Evolution Postdoctoral Fellow, Smithsonian Tropical Research Institute and Scripps Institute of Oceanography
06/94-08/95	Postdoctoral Research Fellow, University of California, Davis

C. RELEVANT PUBLICATIONS

McCartney, M.A., J. Acevedo, C. Rico, E. Bermingham, and W. O. McMillan (in review). Genetic mosaic in a marine species flock. **Molecular Ecology.**

McCartney, M.A. and H.A. Lessios (2002). A quantitative analysis of gametic incompatibility between closely related species of neotropical sea urchins. **Biological Bulletin** 202: 166-181**.**

McCartney, M.A., Keller, G. and H.A. Lessios (2000). Dispersal barriers in tropical oceans and speciation of Atlantic and eastern Pacific *Echinometra* sea urchins. **Molecular Ecology** 9:1391-1400**.**

McCartney, M.A. 1997. Sex allocation and male fitness gain in a colonial, hermaphroditic marine invertebrate. **Evolution** 51:127-140.

Yund, P. O., and M. A. McCartney. 1994. Male reproductive success in sessile invertebrates: competition for fertilizations. **Ecology** 75:2151-2167.

Levinton, J.S., D.E. Martinez, M.A. McCartney, and M.L. Judge. 1995. The effect of water flow on movement, burrowing, and distributions of the gastropod *Illyanassa obsoleta* in a tidal creek. **Marine Biology** 122: 417-424.

D. Current and Pending Support

National Science Foundation. "The blue crab exoskeleton—a model system for studying the control of biomineralization." R.D. Roer, R.M. Dillaman, T.H. Shafer, and M.A. McCartney, $312,873.

National Marine Fisheries Service, Coral Reef Initiative. "Mutton snapper aggregations at Riley's Hump-genetic analysis of upstream aggregations as a potential source of recruits." M.L. Burton and M.A. McCartney, $170,000.

US Fish and Wildlife Service. "A mitochondrial DNA-based assessment of the systematic status of the endemic Waccamaw darter," $4,830.

Center for Marine Science, UNCW. "AFLP-based fingerprinting, and toxin profiling of clonal isolates of the Florida 'red tide' dinoflagellate *Karenia brevis*," $16,200.

NC Department of Transportation (pending). "An evaluation of hemolymph extraction as a non-lethal sampling method for genetic identification of freshwater mussel species in southeastern North Carolina." M.A. McCartney and A. Wilbur, $67,000.

E. Synergistic activities

Courses developed:

- *BIO 206 (Animal Biology)*. Basic course in zoology taught to over 200 students per year. Diversity survey with an evolutionary approach, emphasizing fossil history, coupled to an overview of the comparative anatomy and physiology of representatives of the major animal phyla. Prepared all lectures, exams, and *PowerPoint* presentations.
- *BIO 430/529 (Evolutionary Biology).* Core course in evolution taught to about 25 undergraduates and 12 graduate students per year. A very broad introduction to the field. Scientific writing projects emphasized.
- *BIO 466 (Conservation Biology).* A new course I am teaching in Spring Semester 2003. Combines lectures with computer software-aided class projects on case studies in conservation.
- *BIO 495 (Seminar: Conservation Genetics) and BIO 495 (Seminar: Evolution in Action)*. Senior seminars. I provide foundational lectures and teach students how to prepare and deliver effective *PowerPoint* presentations.
- *Molecular Approaches to Tropical Conservation* (with E. Bermingham (Smithsonian Tropical Research Institute (STRI)) and H. Hollocher (Princeton University)). Taught to about 15 Princeton University undergraduates at STRI.

Outreach efforts:

- I serve as Co-PI with NSF-funded UNCW scientists studying the biochemistry and cell biology of biomineralization in the blue crab, and advise them on state-funded genomics efforts, which involves collaboration with MWG, a biotechnology company.
- I presented seminars at a local museum to advise docents on how to talk to the public about evolution and about the age of the earth.
- I am a "senior project mentor" to Ms. Leticia Locklear from Lakeside, a local high school.
- I served as a science judge at the 2003 National Ocean Bowl, an NSF and NOAA-sponsored high school level academic contest that focuses on the marine sciences.

F. Collaborators and colleagues

Collaborators (Within The Last 48 Months)

D. Levitan (Florida State University); P.O. Yund (University of Maine); D. Baden, R.M. Dillaman, R.D. Roer, T.H. Shafer, A. Szmant (UNC Wilmington); W.O. McMillan (University of Puerto Rico); E. Bermingham, H.A. Lessios (STRI)

Graduate and Postdoctoral Advisors

H.S. Rosenkranz (MS); J.S. Levinton (Ph.D.); R.K. Grosberg, V.D. Vacquier, H.A. Lessios, and D. Levitan (postdoctoral advisors)

Thesis advisees

Thesis advisor for: N. Zhang (graduated 5/01), A. Pogge (graduated 5/02), F. Barreto (current), C. Slaughter (current)
M.S. committee member for: T. Henkel, S. Shehane, A. McElhinney, J. Gabel, E. Buda, P. Kennedy, K. Roman.